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ORIGINAL REPORT

Tumor *PIK3CA* Genotype and Prognosis in Early-Stage Breast Cancer: A Pooled Analysis of Individual Patient Data

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ABSTRACT

Purpose
Phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations are frequently observed in primary breast cancer. We evaluated their prognostic relevance by performing a pooled analysis of individual patient data.

Patients and Methods
Associations between *PIK3CA* status and clinicopathologic characteristics were tested by applying Cox regression models adjusted for age, tumor size, nodes, grade, estrogen receptor (ER) status, human epidermal growth factor receptor 2 (HER2) status, treatment, and study. Invasive disease-free survival (IDFS) was the primary end point; distant disease-free survival (DDFS) and overall survival (OS) were also assessed, overall and by breast cancer subtypes.

Results
Data from 10,319 patients from 19 studies were included (median OS follow-up, 6.9 years); 1,787 patients (17%) received chemotherapy, 4,036 (39%) received endocrine monotherapy, 3,583 (35%) received both, and 913 (9%) received none or their treatment was unknown. *PIK3CA* mutations occurred in 32% of patients, with significant associations with ER positivity, increasing age, lower grade, and smaller size (all $P < .001$). Prevalence of *PIK3CA* mutations was 18%, 22%, and 37% in the ER-negative/HER2-negative, HER2-positive, and ER-positive/HER2-negative subtypes, respectively. In univariable analysis, *PIK3CA* mutations were associated with better IDFS (HR, 0.77; 95% CI, 0.71 to 0.84; $P < .001$), with evidence for a stronger effect in the first years of follow-up (0 to 5 years: HR, 0.73; 95% CI, 0.66 to 0.81; $P < .001$; 5 to 10 years: HR, 0.82; 95% CI, 0.68 to 0.99; $P = .037$); > 10 years: (HR, 1.15; 95% CI, 0.84 to 1.58; $P = .38$; P heterogeneity = .02). In multivariable analysis, *PIK3CA* genotype remained significant for improved IDFS ($P = .043$), but not for the DDFS and OS end points.

Conclusion
In this large pooled analysis, *PIK3CA* mutations were significantly associated with a better IDFS, DDFS, and OS, but had a lesser prognostic effect after adjustment for other prognostic factors.

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INTRODUCTION

Phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations affect the gene corresponding to the alpha isoform of the catalytic subunit (p110) of the class IA kinase and has been extensively studied for its role in human carcinogenesis.[1] These mutations have been reported in a variety of human cancers, including colorectal, endometrial, and ovarian cancer, among others.[2-4] In primary breast cancer (BC), *PIK3CA* mutations are frequent, with the highest frequency among the hormone receptor–positive tumors.[5-7] Approximately 80% of mutations cluster in hot spots located within the helical domain and the kinase domain. The functional consequences of these hot-spot mutations have been studied extensively at the preclinical level, indicating that they are potent mediators of oncogenesis through AKT activation and evasion of apoptosis, as well as induction of an invasive and migratory phenotype.[8-11] In addition, *PIK3CA* mutations have been associated at

ASSOCIATED CONTENT

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the preclinical and clinical level with resistance to paclitaxel, trastuzumab, and endocrine treatment.[9,12-15]

On the basis of the high frequency of *PIK3CA* mutations in BC, as well as the preclinical data supporting their multifaceted oncogenic functions, several studies have assessed their clinical relevance for patients with early-stage disease.[16] In particular, their prognostic relevance has been evaluated with conflicting results.[15] Of note, many of these studies were conducted among heterogeneous patient populations in terms of BC subtypes and treatments received, with some notable exceptions.[17,18] Therefore, in this study, we pooled individual patient data from these previous studies of patients with early-stage BC to robustly evaluate the prognostic associations of these commonly occurring aberrations (and hence their potential relevance for clinical decision making) overall by mutation gene location as well as interactions by BC subtype.

PATIENTS AND METHODS

Types of Studies and Search Strategies

Potentially eligible studies were retrieved through an electronic search on PubMed/MEDLINE using the MeSH terms "breast neoplasm" and "PIK3CA protein, human." The literature search was conducted independently by two investigators (D.Z. and D.F.) in January 2013. Identified studies were eligible for this pooled analysis of individual patient data if they met the following requirements: (1) studies conducted in patients with early-stage BC assessing the *PIK3CA* genotype in primary breast tumor, (2) studies comparing clinical outcomes in association with the *PIK3CA* genotype, and (3) studies published in the English language.

There were no restrictions for inclusion in our study in terms of number of patients included, duration of follow-up, prospective versus retrospective nature of the study, patients' age, menopausal status, BC subtype, or treatment modalities applied. In addition, no restrictions were applied in terms of *PIK3CA* mutational status assessment method. However, studies conducted in the neoadjuvant setting associating the *PIK3CA* genotype with pathologic complete response rate without reporting results of further clinical outcome were excluded from this pooled analysis. Cross-referencing from relevant studies was performed to confirm retrieval of all potentially eligible studies. In terms of study eligibility, final decisions were taken on consensus between the two investigators who performed the research.

The investigators of the eligible trials were contacted and requested to provide individual patient data on (1) baseline characteristics, including patients' demographics and clinicopathologic characteristics; (2) *PIK3CA* genotype and method used; (3) type of (neo)-adjuvant treatment received; (4) clinical outcome, including type and time of event that occurred; and (5) survival status. Individual patient data were used for all analyses, rather than combining results as in the usual types of meta-analyses (Data Supplement; Fig 1).[17-31]

Statistical Analysis

The primary objective of this study was to assess the potential impact of *PIK3CA* mutations on invasive disease-free survival (IDFS), and secondary objectives were to assess the prognostic impact in terms of distant disease-free survival (DDFS) and overall survival (OS).

IDFS was defined as the time from diagnosis until local, regional, or distant recurrence; contralateral BC; second primary malignancy; or death.[32] IDFS analysis time was censored at the last date the patient was known to be alive and recurrence free. DDFS included only distant recurrence and death as events.[32] DDFS analysis time was censored at the date last known to be alive and distant recurrence free. OS included only death as the event; patients were censored at the date last known to be alive.

Differences in patient and tumor characteristics by *PIK3CA* mutation status were assessed using χ^2 tests for categorical variables, the Cochran-Armitage trend test for ordinal variables, and the Wilcoxon rank sum test for continuous variables. Cox proportional hazard models were used to assess associations between *PIK3CA* mutation status and IDFS, DDFS, and OS. Hazard ratios (HRs) and 95% CIs were estimated from univariable and multivariable models. Multivariable models included age (fitted as cubic splines because the effect of age on prognosis is U-shaped), stratified on tumor size (T1/T2/T3/T4), positive nodes (yes/no), local histologic grade (1 to 2/3 to 4), estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) status (positive/negative), and treatment (chemotherapy/endocrine therapy/both). Variables for which the proportional hazards assumption was violated were included as strata rather than covariables. The global test of proportional hazards was not violated for any of the results presented below, unless stated otherwise. We used time intervals defined by the Early Breast Cancer Trialists' Collaborative Group to subdivide the time scale (0 to 5, 5 to 10, and $>$ 10 years).[33] Exploratory objectives were to explore interactions between *PIK3CA* genotype and clinical outcome according to BC subtype (defined using ER and HER2 status), age, and the possible impact of *PIK3CA* genotypes on the timing of recurrence (early *v* late). Median follow-up time was calculated using the reverse Kaplan-Meier method. All data analyses were conducted in R, version 3.1.2.

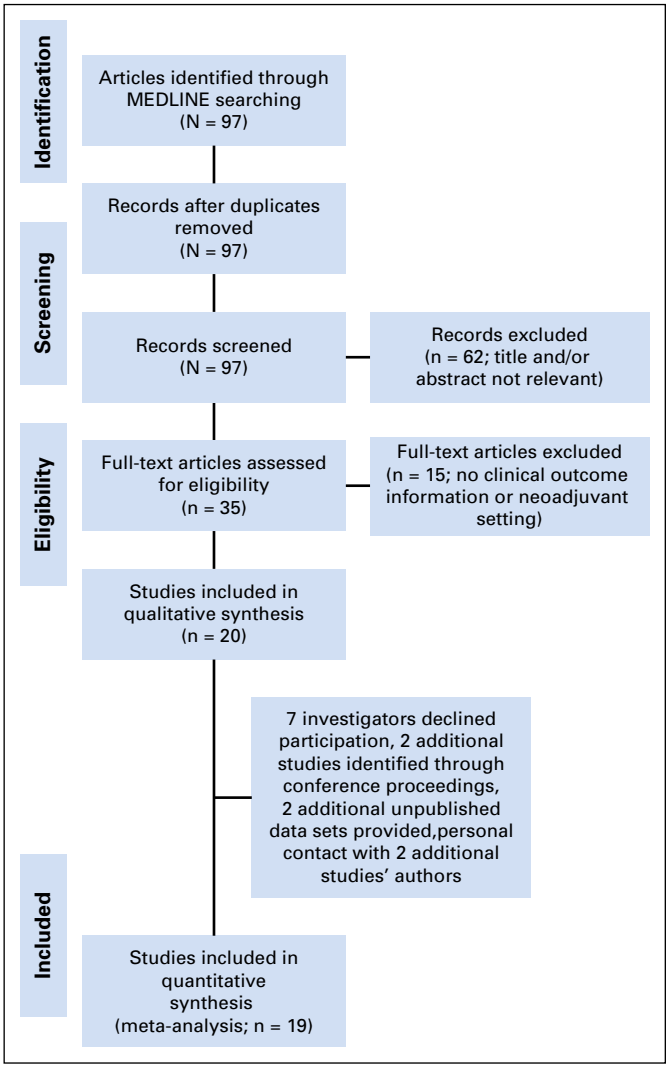

Fig 1. PRISMA study flow chart.

RESULTS

Patient Characteristics

Data on 10,319 patients with known *PIK3CA* genotype originating from 19 studies were available (Fig 1; Data

Table 1. Summary of Patient and Disease Characteristics According to *PIK3CA* Mutation Type

Characteristic	PIK3CA Mutation Status		*P*	All Patients
	Wild Type	Mutant		
Age (continuous; years)			< .001	
Mean (SD)	58.4 (12.5)	61.0 (11.6)		59.2 (12.3)
Median (range)	59 (18-95)	61 (21-96)		59.7 (18-96)
Interquartile range	50-67.3	53-69.1		51-68
Unknown	27	12		39
Age (categorical; years)			< .001	
≤ 50	1,789 (75.1)	594 (24.9)		2,383 (23.2)
> 50	5,222 (66.1)	2,675 (33.9)		7,897 (76.8)
Unknown	27	12		39
Menopausal status			< .001*	
Premenopausal	1,174 (76.5)	361 (23.5)		1,535 (19.0)
Perimenopausal	7 (63.6)	4 (36.4)		11 (0.1)
Postmenopausal	4,230 (65.0)	2,282 (35.0)		6,512 (80.8)
Unknown	1,627	634		2,261
Histology			< .001	
Ductal	4,727 (69.1)	2,111 (30.9)		6,838 (82.2)
Ductal-lobular	137 (57.3)	102 (42.7)		239 (2.9)
Lobular	585 (61.5)	366 (38.5)		951 (11.4)
Other	244 (82.7)	51 (17.3)		295 (3.5)
Unknown	1,345	651		1,996
Local histologic grade (ordered)			< .001†	
Poorly differentiated/undifferentiated	2,752 (76.2)	861 (23.8)		3,613 (41.1)
Moderately differentiated	2,722 (64.0)	1,528 (36.0)		4,250 (48.3)
Well differentiated	487 (52.3)	445 (47.7)		932 (10.6)
Unknown	1,077	447		1,524
Central histologic grade (ordered)			.001†	
Poorly differentiated/undifferentiated	128 (75.7)	41 (24.3)		169 (47.1)
Moderately differentiated	108 (69.7)	47 (30.3)		155 (43.2)
Well differentiated	14 (40.0)	21 (60.0)		35 (9.7)
Unknown	6,788	3,172		9,960
ER status			< .001	
Negative	1,348 (81.7)	301 (18.3)		1,649 (16.1)
Positive	5,627 (65.5)	2,959 (34.5)		8,586 (83.9)
Unknown	63	21		84
PR status			< .001	
Negative	2,329 (76.0)	736 (24.0)		3,065 (33.2)
Positive	3,970 (64.4)	2,196 (35.6)		6,166 (66.8)
Unknown	739	349		1,088
HER2 status			< .001	
Negative	5,120 (65.9)	2,648 (34.1)		7,768 (79.7)
Positive	1,540 (77.7)	441 (22.3)		1,981 (20.3)
Unknown	378	192		570
Subtype			< .001	
HER2 negative/ER negative	790 (82.5)	167 (17.5)		957 (9.8)
HER2 negative/ER positive	4,313 (63.5)	2,475 (36.5)		6,788 (69.8)
HER2 positive	1,540 (77.7)	441 (22.3)		1,981 (20.4)
Unknown	395	198		593
Tumor size (mm)			.001	
Mean (SD)	26.3 (16.1)	25.00 (14.3)		25.90 (15.5)
Median (range)	23 (0-250)	22 (0-180)		22 (0-250)
Interquartile range	16-30	15-30		16-30
Unknown	622 (71.6%)	247 (28.4%)		869
Tumor size (ordered)			< .001†	
T0	1 (16.7)	5 (83.3)		6 (0.1)
T1	2,861 (65.4)	1,511 (34.6)		4,372 (43.2)
T2	3,510 (69.8)	1,516 (30.2)		5,026 (49.7)
T3	423 (74.3)	146 (25.7)		569 (5.6)
T4	92 (63.0)	54 (37.0)		146 (1.4)
Unknown	151	49		200

NOTE. Data are represented as No. (%) unless otherwise noted.
Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; SD, standard deviation.
*Test included premenopausal and postmenopausal only.
†Test for trend.

Table 2. Association of *PIK3CA* Mutations With IDFS, DDFS, and OS

Univariable	PIK3CA Hazard Ratio (95% CI) (MT relative to WT)		
	IDFS	DDFS	OS
All data	0.77 (0.71 to 0.84); $P < .001$* (n = 9,863; 2,433 events)	0.79 (0.72 to 0.86); $P < .001$* (n = 9,697; 2,139 events)	0.90 (0.82 to 0.99); $P = .027$* (n = 9,578; 2,094 events)
0-5 years after diagnosis	0.73 (0.66 to 0.81); $P < .001$ (n = 9,857; 1,749 events)	0.74 (0.66 to 0.83); $P < .001$ (n = 9,695; 1,517 events)	0.89 (0.79 to 1.00); $P = .057$ (n = 9,576; 1,285 events)
5-10 years after diagnosis	0.82 (0.68 to 0.99); $P = .039$ (n = 6,797; 520 events)	0.82 (0.67 to 1.00); $P = .045$ (n = 6,881; 471 events)	0.93 (0.79 to 1.10); $P = .393$ (n = 7,010; 644 events)
> 10 years after diagnosis	1.15 (0.84 to 1.58); $P = .380$ (n = 1,045; 160 events)	1.18 (0.85 to 1.63); $P = .323$ (n = 1,032; events = 151)	0.87 (0.62 to 1.20); $P = .383$ (n = 927; 165 events)
Multivariable†	**IDFS**	**DDFS**	**OS**
All data	0.88 (0.78 to 1.00); $P = .043$ (n = 6,120; 1,417 events)	0.88 (0.77 to 1.00); $P = .054$ (n = 5,919; 1,245 events)	0.98 (0.86 to 1.12); $P = .799$ (n = 5,730; 1,184 events)
0-5 years after diagnosis	0.87 (0.75 to 1.00); $P = .048$ (n = =6,120; 1,020 events)	0.85 (0.73 to 0.99); $P = .042$ (n = 5,919; 891 events)	0.99 (0.83 to 1.16); $P = .869$ (n = 5,730; 718 events)
5-10 years after diagnosis	0.88 (0.68 to 1.13); $P = .324$ (n = 4,300; 307 events)	0.92 (0.70 to 1.21); $P = .548$ (n = 4,328; 268 events)	0.99 (0.79 to 1.24); $P = .911$ (n = 4,354; 384 events)
> 10 years after diagnosis	1.10 (0.64 to 1.88); $P = .740$ (n = 691; 90 events)	1.04 (0.60 to 1.81); $P = .886$ (n = 684; 86 events)	0.91 (0.49 to 1.68); $P = .764$ (n = 585; 82 events)

Abbreviations: DDFS, distant disease-free survival; IDFS, invasive disease-free survival; MT, mutated; OS; overall survival; WT, wild type.
*Proportional hazard assumption violated (IDFS: $P < .001$; DDFS: $P < .001$; OS: $P = .27$).
†Adjusted for age (fitted as cubic spline) and stratified on tumor size (T1/T2/T3/T4), positive nodes (yes/no), local grade (poorly differentiated or undifferentiated/moderately differentiated/well differentiated), estrogen receptor (positive/negative), human epidermal growth factor receptor 2 (positive/negative), and treatment (chemotherapy/hormone therapy/both).

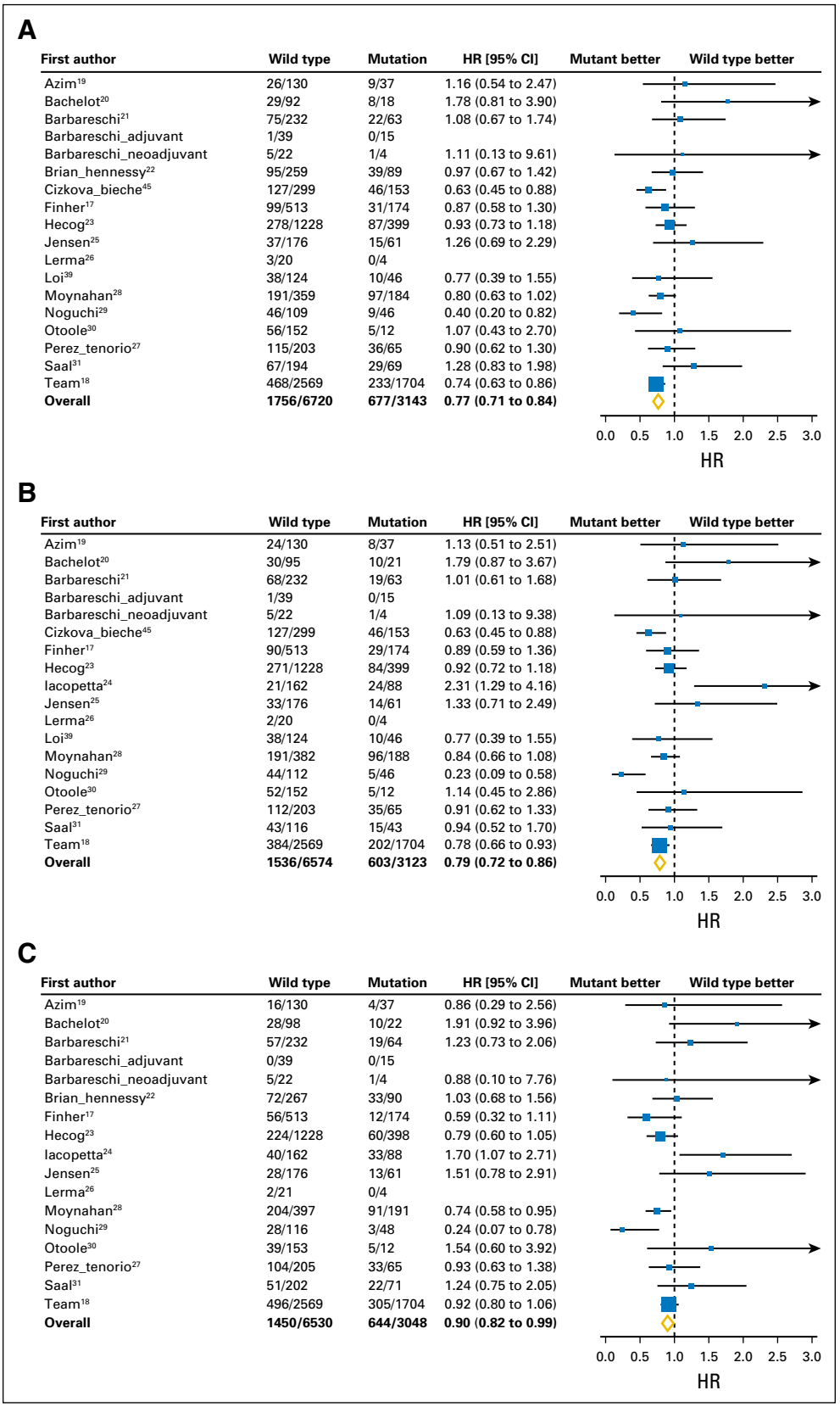

Fig 2. Unadjusted effect of *PIK3CA* genotype on invasive disease-free survival (IDFS), distant disease-free survival (DDFS), and overall survival (OS) by study and for all pooled data by data set. HR, hazard ratio.

Supplement). Overall, the median age at diagnosis was 60 years (range, 18 to 95), and the median follow-up time was 6.9 years (range, 2 days to 21.5 years); 1,787 patients (17%) received chemotherapy, 4,036 (39%) received endocrine treatment, 3,583 (35%) received both, and 895 (9%) received none or the treatment was unknown.

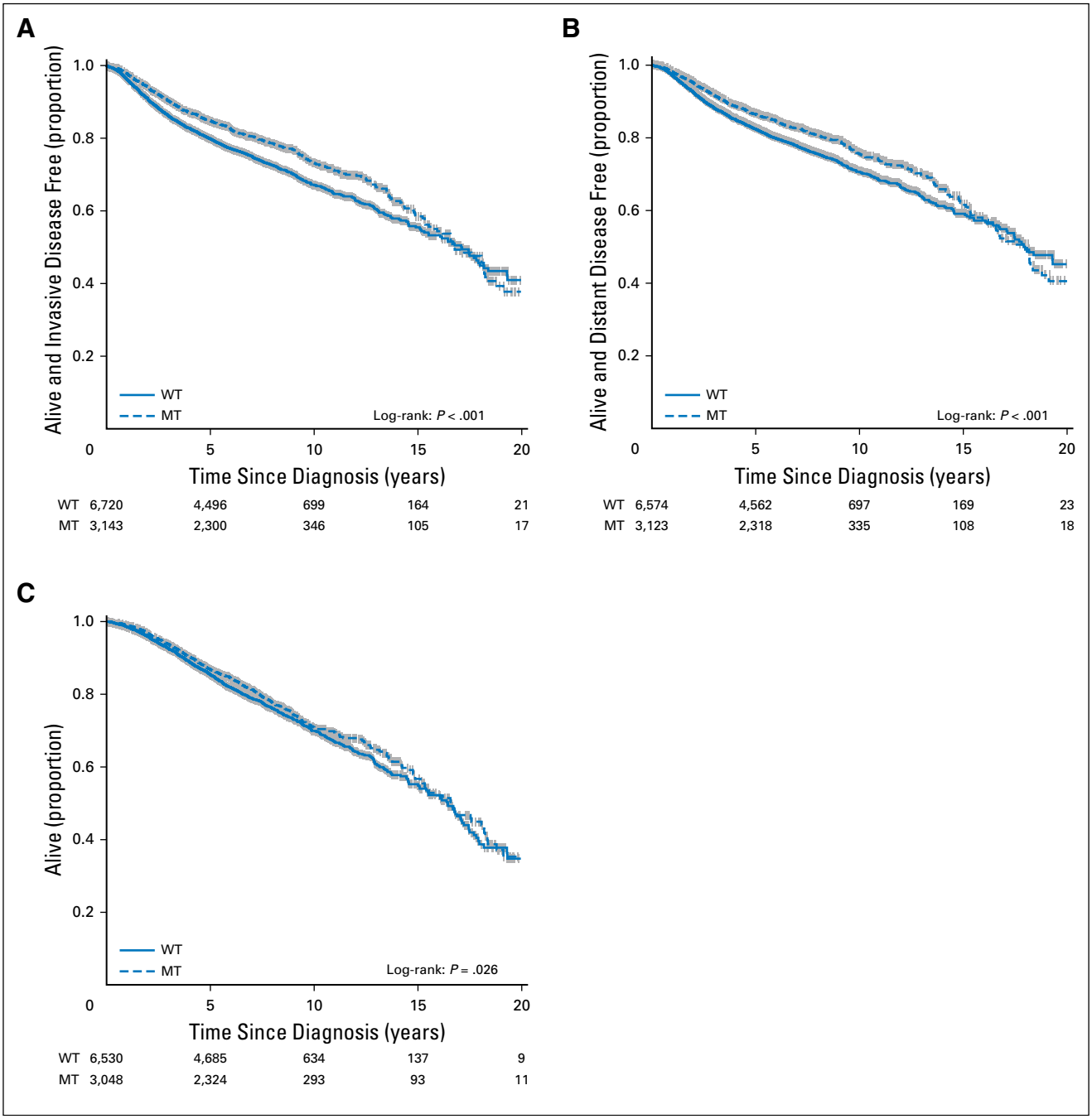

Fig 3. Kaplan-Meier curves according to *PIK3CA* genotype for (A) invasive disease-free survival, (B) distant disease-free survival, and (C) overall survival using all pooled data. MT, mutated; WT, wild type.

Associations Between PIK3CA *Mutations With Clinicopathologic Variables*

Summary results from the comparison of baseline characteristics and *PIK3CA* mutation status are listed in Table 1. *PIK3CA* mutations were identified in the tumors of 3,281 patients (32%); 1,705 (52%) of these were in the kinase domain and 1,263 (39%) were in the helical domain. *PIK3CA* mutations were more common in older patients, ER-positive tumors, and lower-grade and smaller tumors. The frequency of *PIK3CA* mutations also differed significantly by BC subtypes defined by combined ER and HER2 status (HER2-negative/ER-negative [also known as triple-negative breast cancer], 18%; HER2-negative/ER-positive [also known as luminal], 37%; HER2-positive, 22%; $P < .001$).

Associations Between PIK3CA *Mutations and Prognosis*

In the univariable analysis, *PIK3CA* mutations were significantly associated with better IDFS (HR, 0.77; 95% CI, 0.71 to 0.84; $P < .001$; Table 2). Figure 2 shows the estimated HRs and 95% CIs for each of the studies individually and for all data combined for IDFS, DDFS, and OS. No significant heterogeneity was found

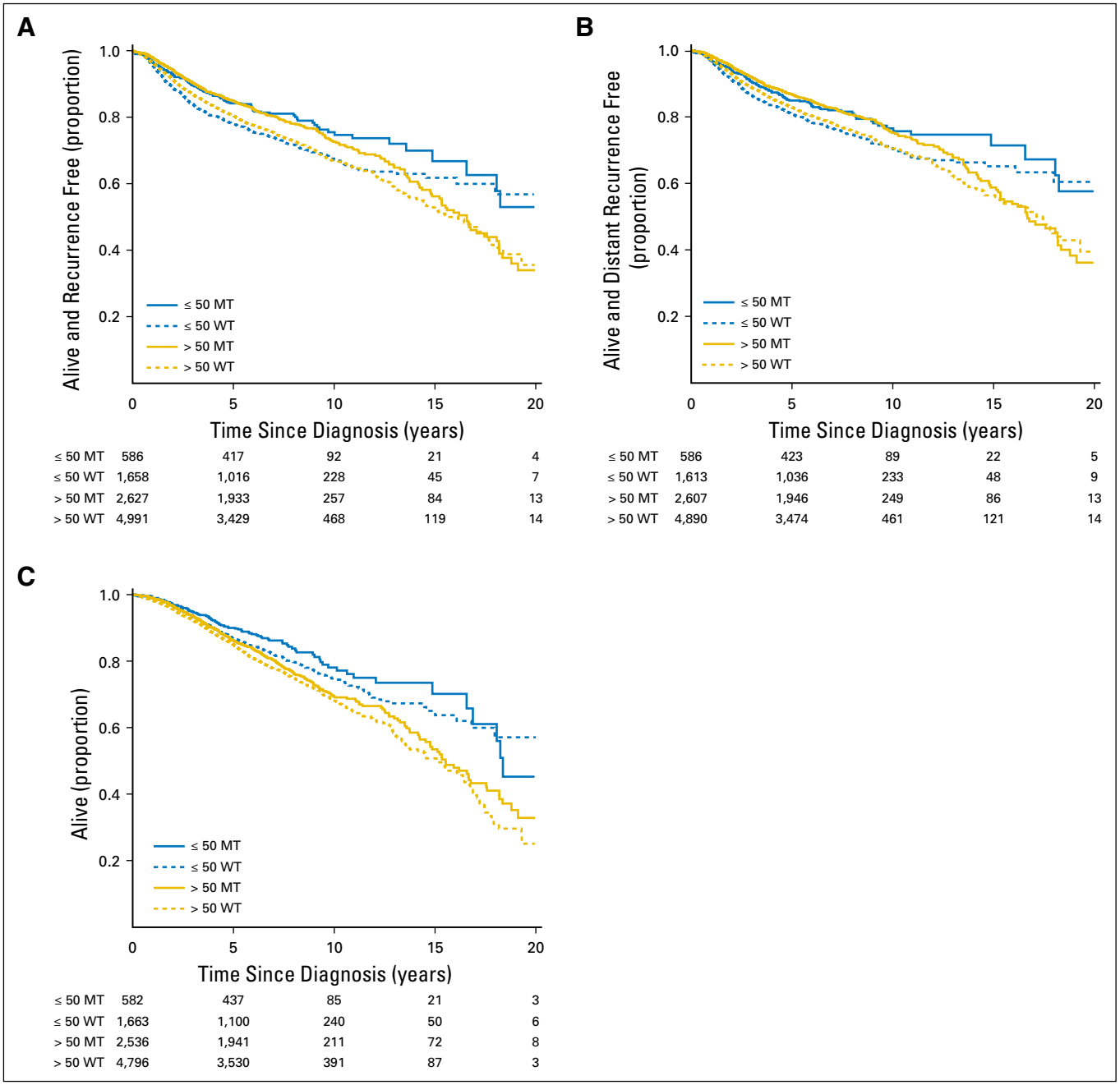

Fig 4. Kaplan-Meier curves for (A) invasive and (B) distant disease-free survival and (C) according to *PIK3CA* genotype and age category. MT, mutated; WT, wild type.

between studies in the univariable effect of *PIK3CA* mutation status on IDFS (*PIK3CA* genotype by study interaction; $P = .12$). For both DDFS and OS, significant heterogeneity was found between studies ($P = .004$ and $P = .009$, respectively). Evidence of departure from the proportional hazards assumption was observed ($P < .001$), implying that the effect of *PIK3CA* genotype on IDFS changed over time. Patients with *PIK3CA* mutant tumors had better IDFS during the first 10 years after diagnosis (0 to 5 years: HR, 0.73; 95% CI, 0.66 to 0.81; $P < .001$; 5 to 10 years: HR, 0.82; 95% CI, 0.68 to 0.99; $P = .037$) but not after 10 years (HR, 1.15; 95% CI, 0.84 to 1.58; $P = .38$; P heterogeneity = .02; Table 2; Fig 3).

There was similar evidence of a nonproportional hazard of relapse over time for DDFS ($P < .001$), although not for OS ($P = .27$). Of note, the nonproportionality was significant only when all BC subtypes were combined (Data Supplement).

After adjusting for age, tumor size, nodal status, local grade, ER status, HER2 status, and treatment, *PIK3CA* status remained significant for IDFS (HR, 0. 88; 95% CI, 0.78 to 1.00; $P = .043$) but not for DDFS ($P = .054$) and OS ($P = .8$; Table 2).

The effects of *PIK3CA* genotype on the IDFS and DDFS by BC subtype can be found in the Data Supplement. Notably, there were no significant interactions observed between *PIK3CA* mutation status, BC subtype, and prognosis with the exception of HER2

disease and OS (IDFS: $P = .16$; DDFS: $P = .39$; OS: $P = .04$; Data Supplement) where *PIK3CA* mutations were associated with a worse OS. Mutation location (helical *v* kinase domain) also did not seem to significantly affect prognosis (IDFS: $P = .74$; DDFS: $P = .92$; OS $P = .65$; Data Supplement). In an exploratory analysis, a significant interaction between *PIK3CA* status and continuous age at diagnosis was observed for IDFS and OS but not for DDFS (IDFS: $P = .032$; DDFS: $P = .20$; OS: $P < .001$; shown in Figure 4 using categorized age at 50 years) where younger patients with a mutation had better survival.

DISCUSSION

The potential prognostic relevance of *PIK3CA* mutations in early-stage BC has thus far been unclear. The larger data sets have reported *PIK3CA* mutations as a favorable aberration, associated with a better clinical outcome, seemingly somewhat at odds with the notion of *PIK3CA* being considered an oncogenic driver.[18] Our study, pooling data from 19 cohorts reaching a total of 10,319 patients, confirms this finding in the univariable analyses. *PIK3CA* mutations were found to be associated with improved IDFS rates (HR, 0.77; $P < .001$) in the univariable analysis, but this effect was less strong in the multivariable model because of its association with favorable clinicopathologic characteristics, namely, older age, ER positivity, lower grade, and smaller tumor size. Overall, our data did not reveal a consistent difference in its prognostic effect according to BC subtype, with the exception of HER2-positive disease and the end point of OS.

Preclinical evidence indicates possible biologic differences between *PIK3CA* mutations affecting the helical or the kinase domain.[34,35] In particular, mutations of the helical domain have been associated with a more aggressive phenotype.[36] In our pooled analysis, including 1,263 and 1,705 patients with *PIK3CA* mutations in the helical and kinase domain, respectively, we found no significant differences in their prognostic impact, similar to studies previously reported.[18,23]

Associations with ER positivity remain intriguing. Tikoo et al[37] reported an increase in the luminal progenitor population in their *PIK3CA* knock-in mouse model, suggesting that *PIK3CA* mutation was important in BC initiation.[37] This is supported by the observation that *PIK3CA* mutations exist at high frequency in DCIS.[38] Loi et al[39] reported decreased mTORC1 signaling as well as upregulation of ER-related genes at the gene expression level in *PIK3CA* mutant ER-positive primary BCs. These data suggest that *PIK3CA* mutations could drive oncogenesis through ER signaling.[39] Alternatively, *PIK3CA* mutations have been associated with the induction of senescence in BC, with similar findings reported in other tumor histologies.[40,41] Hence, despite being a known driver mutation, *PIK3CA* mutations seem to contribute to a favorable clinicopathologic phenotype and behave less aggressively than BCs with driver gene amplifications. These observations should be distinguished from reports that PI3K pathway activation per se is associated with endocrine therapy resistance.[42]

Of note, we confirm a previous observation that the positive prognostic relevance of *PIK3CA* mutations is nonproportional, that is, the strongest effect is in the first 5 years and decreases over time. The explanation for this remains unclear.[17] However, we note that the proportion of patients with over 10 years of follow-up is < 10% and critically does not exclude (lower bound of HR, 0.84) better IDFS; therefore, the departure from proportional hazards could be due to either a reduction in benefit over time or a loss of this effect, or bias in patients with longer follow-up. Taking into account the natural history of BC and the latency of the disease, with relapses occurring even after considerable time after the primary diagnosis, the median follow-up of patients in our pooled analysis (6.9 years) should be taken into consideration when interpreting the results. We also observed a significant interaction between age and *PI3KCA* mutation status. It would be valuable to further validate this finding in adjuvant endocrine studies of premenopausal women.[43]

Our study has significant strengths, namely, that it is the largest to date performed in patients with early-stage BC using large data sets derived from prospective randomized clinical trials with the use of individual patient-level data. Of note, the size of this data set enabled us to assess the prognostic relevance of *PIK3CA* mutations across all subtypes of BC, and the use of prospective clinical trial data sets may overcome some biases inherent in retrospective institutional series. We acknowledge the limitations: (1) significant heterogeneity among data sets; (2) the treatments administered did not follow a set protocol; in particular, some of the patients with HER2-positive BC did not receive (neo)adjuvant trastuzumab-based treatment, and some of the treatments administered differed from current standards (Data Supplement); (3) only articles published in English were included; (4) only published studies were included (ie, possible bias toward positive results); and (5) heterogeneity in terms of methods of assessment of *PIK3CA* mutation status.

In summary, our results indicate that *PIK3CA* somatic mutations are associated with a significantly better clinical outcome in the univariable but to a lesser extent in the multivariable analysis in early-stage BC. Next-generation sequencing studies have reported that *PIK3CA* mutations often coexist with other genetic alterations.[44] Integration of coexistent genetic alterations and, potentially, plasma analyses and other markers of PI3K pathway activation will better refine prognostic assessments of *PIK3CA* mutant early-stage BC patients.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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Financial support: Sherene Loi
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